Validation of a Light Aspiration device for in vivo Soft TIssue Characterization (LASTIC)

Vincent Luboz, Emmanuel Promayon, Grégory Chagnon, Thierry Alonso, Denis Favier, Christine Barthod, Yohan Payan

Validation of a Light Aspiration device for in vivo Soft TIssue Characterization (LASTIC)

Vincent Luboz[1], Emmanuel Promayon[1], Grégory Chagnon[2], Thierry Alonso[2], Denis Favier[2], Christine Barthod[3], Yohan Payan[1]

[1] Laboratoire TIMC-IMAG, équipe GMCAO, Université Joseph Fourier - CNRS, 38706 La Tronche cedex, France. {epromayon, vluboz, ypayan}@imag.fr

[2] Laboratoire 3SR, Université Joseph Fourier - CNRS, BP 53, 38041 GRENOBLE cedex 9, France. {gregory.chagnon, denis.favier}@grenoble-inp.fr, thierry.alonso@ujf-grenoble.fr

[3] Laboratoire SYMME, Université de Savoie, BP 80409, 74944 Annecy le Vieux cedex, France. christine.barthod@univ-savoie.fr

Abstract In-vivo characterization of soft tissue is a key step towards accurate biomechanical simulation enabling planning and intra-operative assisted surgery. This chapter presents the new version of LASTIC, a device measuring soft tissue deformations using a negative pressure. Its capabilities are compared with standard tensile tests on five samples with different elastic properties, i.e Young modulus, from 10 kPa to 1 MPa, in order to estimate its accuracy and define the functional measurement range. Results show that LASTIC overestimates Young modulus by an average of 24 % compared to the tensile devices. This error, although rather large, allows a first estimation of the elastic modulus of different materials, especially living tissues, even during surgery. Directions for improvements are given that will allow for better patient-specific biomechanical simulations of soft tissues.

Keywords: soft tissue characterization, aspiration, mechanical behavior, inverse analysis, planning and assistance.

1 Introduction

Living soft tissues are known to exhibit non-linear, inhomogeneous, anisotropic, patient-specific, time and rate-dependent behaviors. Taking into account such specificities is highly challenging, especially for intra-operative computer aided devices that require an on-line estimation of the constitutive behavior of the tissues.

This chapter introduces the Light Aspiration device for in vivo Soft TIssue Characterization (LASTIC) which principles were proposed by our group and used, in its first version, to evaluate the constitutive behavior of skin (Schiavone et al., 2007), tongue (Schiavone et al., 2008) and brain tissues (Schiavone et al., 2009). In its first version the device was based on the *pipette aspiration* principle which consists in measuring the tissues deformations induced by a negative pressure. The surgeon held the instrument and established contact with the tissues surface while the device measured the negative pressures and displacement responses. This implied that (1) the device had to undergo a full sterilization and (2) the data processing to be sufficiently fast to provide an interactive estimation of the tissues constitutive equation. These two constraints have encouraged us to propose a new version of LASTIC (Schiavone et al., 2010). This chapter aims at validating this new version by comparing, for synthetic materials, the constitutive parameters it estimates with the parameters provided by usual tensile test machines. This validation is indeed a prerequisite before any intra-operative use of the new version of LASTIC.

Next section introduces the samples and the devices that were used to estimate the materials' mechanical parameters. The remaining provides the corresponding results (section 3) before a discussion (section 4) and a conclusion (section 5).

2 Material and methods

2.1 Elastic samples

Although human soft tissues are considered to be nonlinear viscoelastic, most of the work done to simulate them analyzes their behavior on the linear elastic side. To provide a better coverage of human soft tissues' elasticity and behavior, experiments were conducted on five different samples. Their elastic moduli ranged from few kilopascals to several hundred kilopascals (kPa thereafter). According to the literature, several materials correspond to this elasticity. We therefore chose to use the following ones:

- RTV#1: a RTV-EC00 silicone (artificina.com), made from a mix of 50 % of base and 50 % of catalyst (also called curing agent). It has a linear behavior up to 25 % of engineering strain (see Figure 6).
- RTV#2: the same RTV-EC00 silicone, but made from a mix of 40 % of base and 60 % of catalyst, to create a softer silicone than RTV#1. It has a linear behavior up to 25 % of engineering strain (see Figure 6).

- RTV#3: a RTV 141 silicone (artificina.com), consisting of 90 % of base and 10 % of catalyst. It has a linear behavior up to 120 % of engineering strain, where rupture of the material occurs.
- Ecoflex: an Ecoflex® 00-30 silicone (Smooth-on.com) constituted by two bases mixed in equal proportion. It has a linear behavior up to 15 % of engineering strain (see Figure 6).
- Candle gel: a gel (glorex.com) that is fluid at temperatures over 95 °C. It is fragile at room temperature and tends to tear. It has a linear behavior up to 10 % of engineering strain (see Figure 6).

For each material, two samples were created by two molds in order to perform two different tests: one to be used by LASTIC and one to be used for the tensile tests, see subsections 2.2 and 2.3 for details on the mold shapes. This process ensured that the same material mix is poured in the two molds so that the elasticity measurements are done on the same material in each testing machine. Each test is done at least three times on each sample to take the variability of the measurements of the elastic modulus into account, except for the RTV#3 which was extensively characterized in previous studies (Meunier et al. 2008).

RTV#1, RTV#2 and RTV#3 were all created following the same process: first the carefully weighted amount of base and catalyst were mixed in a container according to the proportion described previously, then the mix was exposed to vacuum for several minutes (about five minutes for RTV#1 and RTV#2, and around 30 minutes for RTV#3) in order to evacuate any air bubbles. The mixes were then poured in the two different molds. The silicones RTV#1 and RTV#2 cured in two hours at room temperature while the RTV#3 cured in 24 hours, including three initial hours at 150° C.

The Ecoflex silicone is created from its two bases by mixing them in a container and allowing it to cure for four hours at room temperature. The mix is also submitted to a vacuum chamber. Five minutes seemed enough to evacuate any air bubbles. Its properties are stationary 24 hours later.

The candle gel is heated to 95° C in a boiling water bath. Once liquefied, it is poured in the two molds and becomes an elastic solid in few minutes. Because of its fragility, precautions must be taken when extracting it out of the mold.

2.2 LASTIC

Based on a prototype aspiration device quantitatively evaluated during surgery on the brain (Schiavone et al. 2009) and during more classical experiments on other organs such as tongue, cheeks and forearm skin, we designed a more elaborate device, called LASTIC (Light Aspiration device for in vivo Soft TIssue Characterization) (Schiavone et al. 2010). LASTIC is intended to be used in operating rooms and therefore to meet the very rigorous sterilization and handling process imposed during surgery. The basic design is not very far from the one proposed by (Vuskovic 2001), except that it is more compact, see Figure 1.

LASTIC is built in a very compact metallic cylinder of 33 mm in height and 34 mm in diameter. This cylindrical case is divided in two compartments. The lower compartment is an airtight cylindrical chamber, open at the bottom by a circular aperture and closed at the top by a glass window. The upper compartment holds the electronic part consisting of a miniature digital camera and a LED used as a light source. The camera is a 9.5 mm × 9.5 mm 2 megapixels mobile phone camera sensor (model VS6750, STMicroelectronics, Geneva, Switzerland) with a resolution of 1600 × 1200 pixels (UXGA).

The complete setup is composed of a programmable syringe pump (Aladdin AL-1000, World Precision Instruments, Inc., FL, USA), a USB digital manometer (model 8215, AZ Instrument Corp, Taiwan) and LASTIC, all linked by Luer-Lok connected flexible medical tube, see Figure 2.

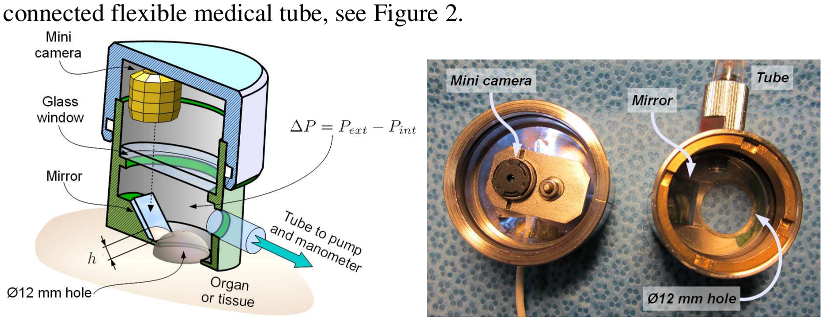


Figure 1 – Cross section of LASTIC: the upper part contains the camera while the lower part is the aspiration chamber with the mirror (left). View of the two chambers when separated (right).

While the lower compartment is in contact with the tissue, a negative pressure ΔP is generated by a software controlled syringe pump programmed to withdraw. The soft-tissue is consequently aspired inside the chamber, where the deformation, measured as the aspiration height, is imaged by the digital camera, by means of a 45° inclined mirror (see Figure 3). The pressure from the manometer is acquired synchronously. Once the micro-leaks, occurring at the interface between the decompressing chamber and LASTIC, are sealed off by a sufficient minimal negative pressure and LASTIC weight, the measurements can be carried out.

The height of the aspired tissue is then segmented on the image and a basic camera calibration is performed to determine the pixel size. The experiment is performed for increasing level of negative pressure. A displacement height is therefore obtained for each applied negative pressure.

We only considered the apex displacements higher than 0.5 mm, which corresponds to the position of the bottom of the mirror relatively to the initial tissue surface. If this level is not reached, there is a risk of measuring a point lower than the deformation dome but appearing higher on the mirror, due to an incorrect inclination of the setup.

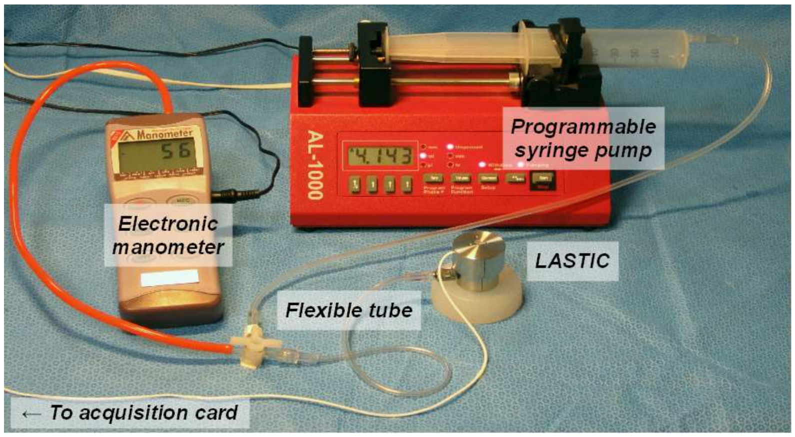


Figure 2 – Complete set up for a measurement: LASTIC is on the silicone sample (here the RTV#2), the manometer is on the left, and the pump is at the top. Luer-Lok connected flexible medical tubes link the three components. They are also all connected to the PC (not shown) that commands the synchronized acquisition.

For the present experiments, the mold produces a material sample of cylindrical shape of approximately 20 mm of height, 60 mm of diameter.

A Finite Element Analysis of the aspiration experiment using a Neo-Hookean constitutive law (Treloar 1943) is used in order to build a library of displacement heights. The Neo-Hookean equation is written as:

$$W = C_1(I_1-3) \quad (1)$$

where I_1 is the first invariant of the left Cauchy-Green deformation tensor, C_1 is a material parameter, and W is the strain energy.

The library of displacements is basically a double entry table depending on C_1 and the applied negative pressure. From this library, a least-square minimization method is used to find the value C_1 in the library that best matches the measurements. This is performed in interactive time (less than 1 s).

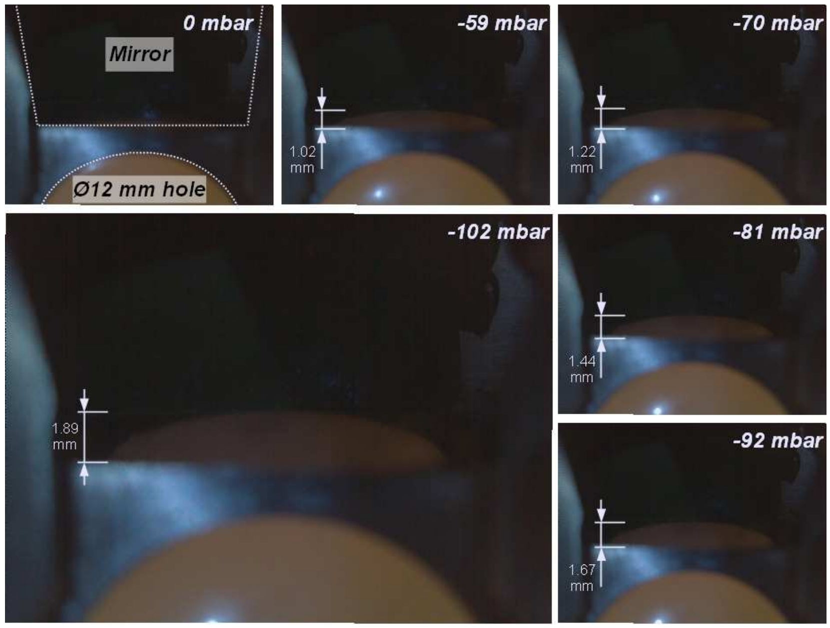


Figure 3 – Images acquired during LASTIC measurements on RTV#2 at different negative pressure values from 0 mbar to -102 mbar. The deformation undergone by the sample can be observed as reflected on the mirror and is measured as the height of the top of the dome.

2.3 Tensile devices

Because it is a well-established characterization technique, tensile test measurements are used as a reference to compare with LASTIC measurements and estimate the resulting precision on the material parameter. Two extension devices were used for the tensile measurements in two different laboratories.

The first tensile device is an Instron® machine (Instron, Norwood, MA, USA), used in the SYMME Laboratory, see right panel of Figure 4. It applies a given displacement to the material and records the force needed to reach it. The material sample had a rectangular shape of 60 mm of length, 15 mm of width and 7 mm of thickness. It was fixed by tensile jaws on each side of its length.

The second extension device is an Eplexor 500N testing device (Gabo Qualiter Testanlagen GmbH, Ahlden/Aller, Germany), used in the 3SR laboratory with a 25 N sensor. It works similarly to the Instron machine: applying a given displacement and recording the relative force. The material sample was also fixed between two tensile jaws, at each extremity, see left panel of Figure 4. It had a rectangular shape of 25 mm of length, 7 mm of width and 2 mm of thickness.

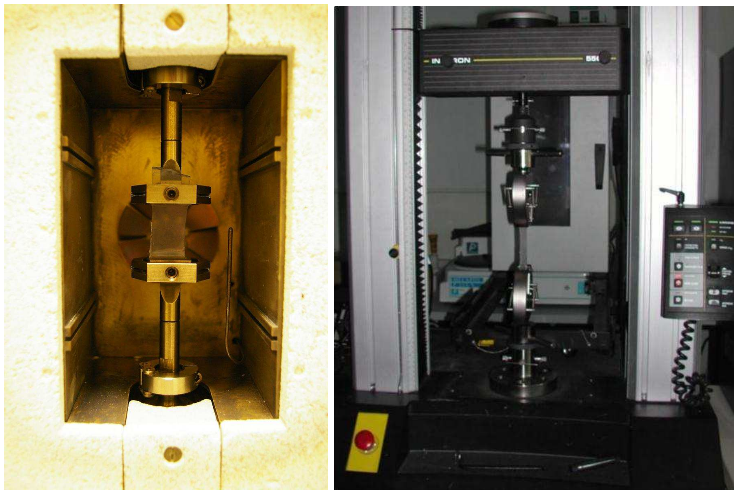


Figure 4 – The Eplexor 500N tensile device applying the displacement load to the RTV#2 silicone (left). Instron® tensile device applying the displacement load to the Ecoflex silicone (right).

To evaluate the elastic modulus from the measurements of both devices, the tensile test stress/strain values are studied by fitting a Neo-Hookean constitutive equation:

$$\pi_{11} = 2C_1(\lambda - 1/\lambda^2) \tag{2}$$

where π_{11} is the First Piola-Kirchhoff stress, C_1 is a material parameter, and λ is the principal stretch. The strain energy density function is the same as Equation (1).

During infinitesimal deformations, one can assume that the deformed and initial configurations coincide. The Cauchy stress can consequently be written as:

$$\sigma_{11} = \pi_{11}\ \lambda \tag{3}$$

As the elongation λ is equal to $1+\varepsilon$ where ε is the engineering strain (measured by the testing device),

$$\sigma_{11} = 2C_1(\lambda^2 - 1/\lambda) = 2C_1((1+\varepsilon)^2 - 1/(1+\varepsilon)) = 6C_1\varepsilon + o(\varepsilon) \tag{3}$$

We deduce from this equation the link between the C_1 parameter and the Young modulus $E = 6C_1$.3 Results

The results of LASTIC measurements on RTV#1, RTV#2, Ecoflex and candle gel are presented in Figure 5 a), b), c) and d) respectively.

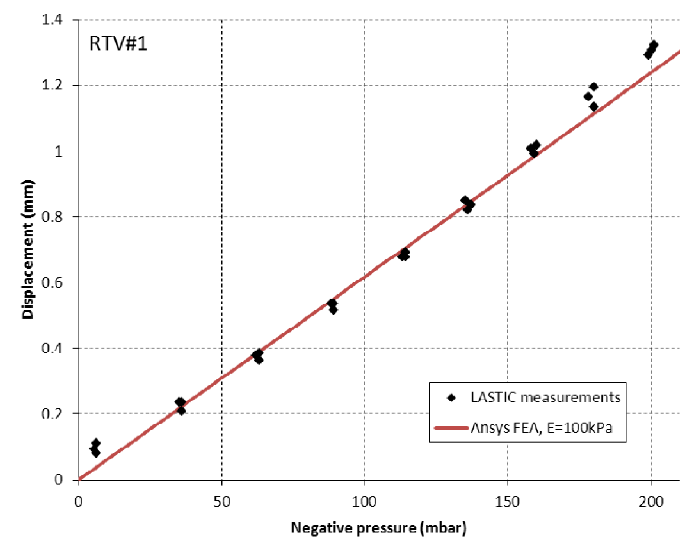
For the RTV#1 sample, the minimal apex displacement, or aspiration height, was reached with a negative pressure of 50 mbar. In total, a volume of 10 ml of air was aspirated with LASTIC, resulting in a negative pressure of nearly 200 mbar and an aspiration height of slightly more than 1.3 mm. A Young modulus of 100 kPa was estimated for this material.

For the RTV#2 sample, a negative pressure of 30 mbar was needed to reach enough apex displacement. In total, a volume of 5ml was pulled with LASTIC, resulting in a negative pressure of about 95 mbar and an aspiration height around 2 mm. A Young modulus of 32 kPa was estimated for this material.

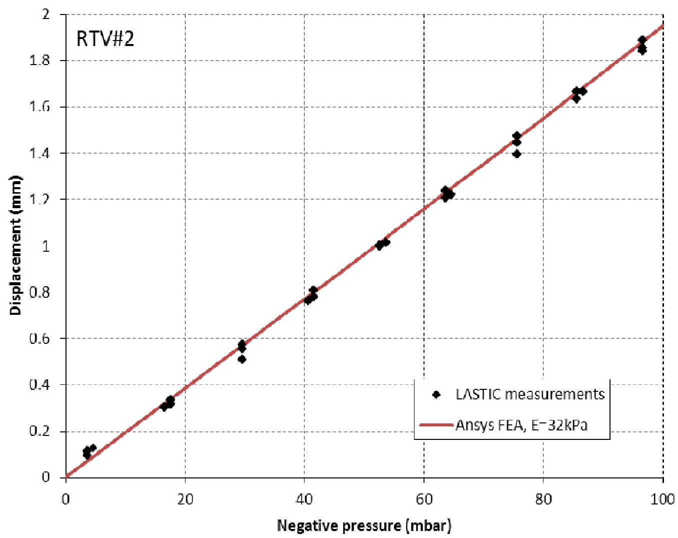
For the RTV#3 sample, the minimum negative pressure could not be reached even though a volume of 60 ml was aspired with the pump, resulting in a negative pressure of about 745 mbar and an apex displacement of approximately 0.4 mm. A higher negative pressure could not be obtained. Because of the stiffness of this material and the high negative pressure needed to deform it, the aspiration tests were unsuccessful. No Young modulus was therefore estimated for this material.

For the Ecoflex sample, a negative pressure of 60 mbar was needed to reach the minimal aspiration height. In total, a volume of 5 ml was aspirated, resulting in a negative pressure of about 140 mbar and an apex displacement close to 1.5 mm. A Young modulus of 67.5 kPa was estimated for this material.

For the candle gel sample, a minimum negative pressure of about 5 mbar was needed. In total, a volume of 5 ml of air was aspirated, resulting in a negative pressure of nearly 50 mbar and an aspiration height of slightly less than 2.2 mm. A Young modulus of 14.5 kPa was estimated for this material.



(a)


RTV#2
Displacement (mm)
Negative pressure (mbar)
LASTIC measurements
Ansys FEA, E=32kPa
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
20
40
60
80
100

(b)

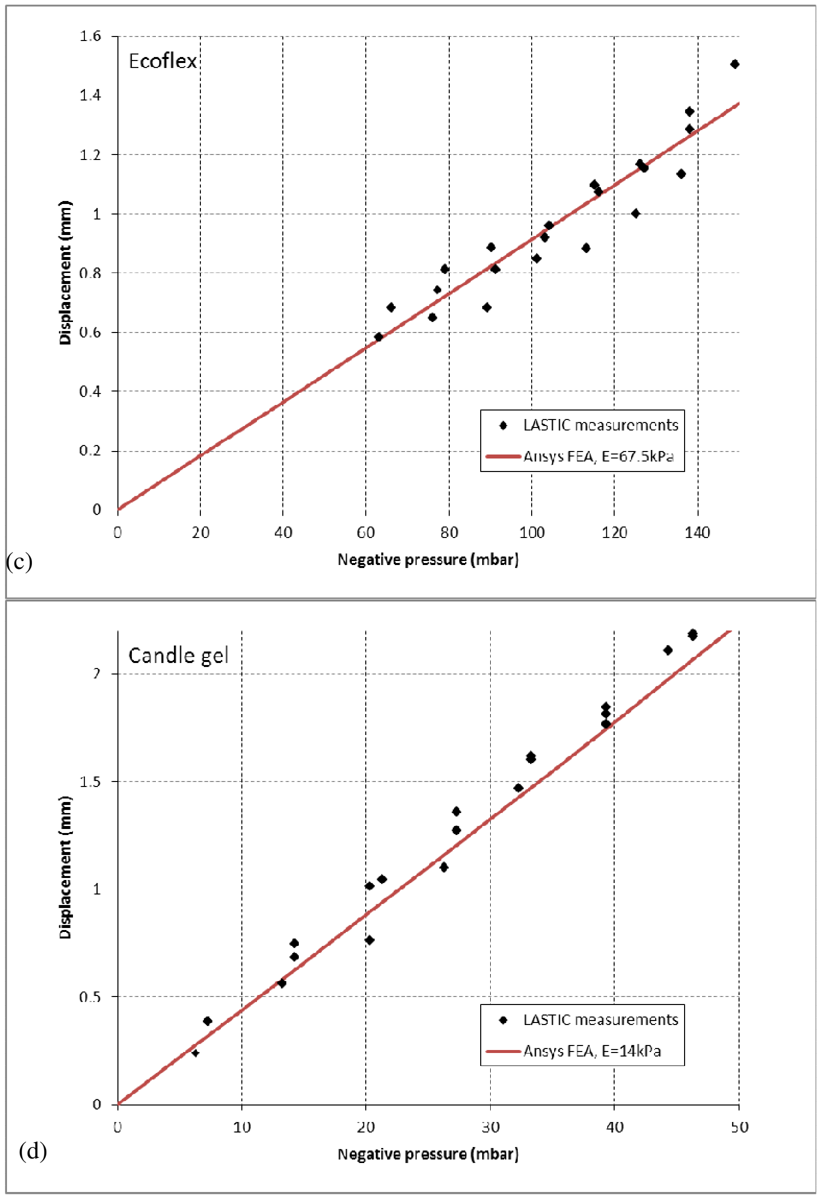


Figure 5 – LASTIC measurements and the corresponding Young modulus estimated by the FE analysis for (a) RTV#1, (b) RTV#2, (c) Ecoflex, and (d) candle gel.

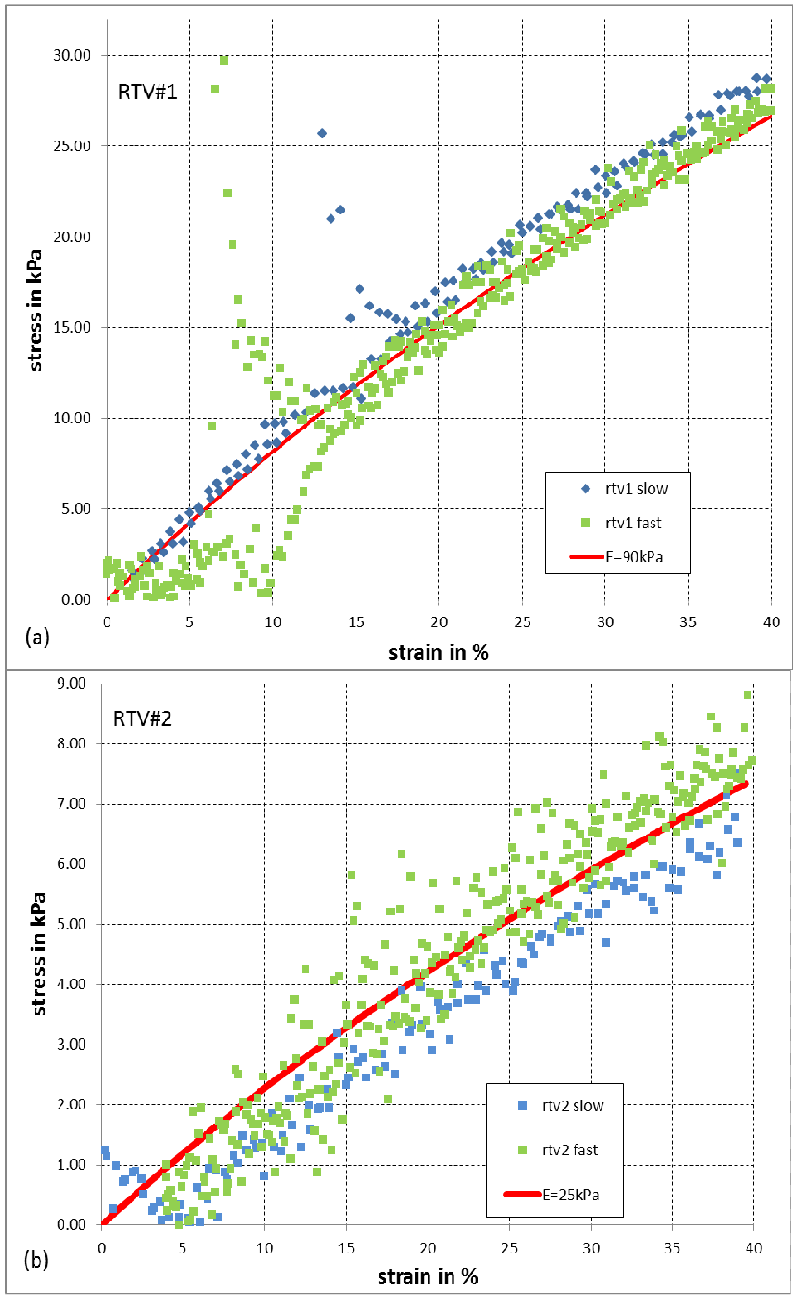
Figure 6 shows the measurements made with the two tensile devices. RTV#1, RTV#2 and RTV#3 were tested with the Eplexor 500N Gabo device while the Ecoflex and the candle gel were tested with the Instron machine. Each sample was placed between the tensile jaws before being deformed in extension. A Neo Hookean curve was then fitted on the measurements to estimate the best Young modulus as explained previously. The results of the tensile device measurements on RTV#1, RTV#2, RTV3, Ecoflex and candle gel are presented in Figure 6 a), b), c), d) and e) respectively.

The RTV#1 sample was pulled at two different strain rates to estimate the influence of viscosity on this silicone. The "slow" tensile test was performed at approximately 3 $mm.min^{-1}$ while the "fast" tensile test was performed at 40 $mm.min^{-1}$. No significant difference was recorded between the two measurements: this silicone had no strain rate dependence in this range of strain rates. A Young modulus of 90 kPa was estimated for this sample, using the Neo Hookean fitting. At the first stage of these tensile tests, some high stresses were locally recorded (visible as two spikes on Figure 6 a), they were probably due to some initial sliding of the sample in the tensile jaws.

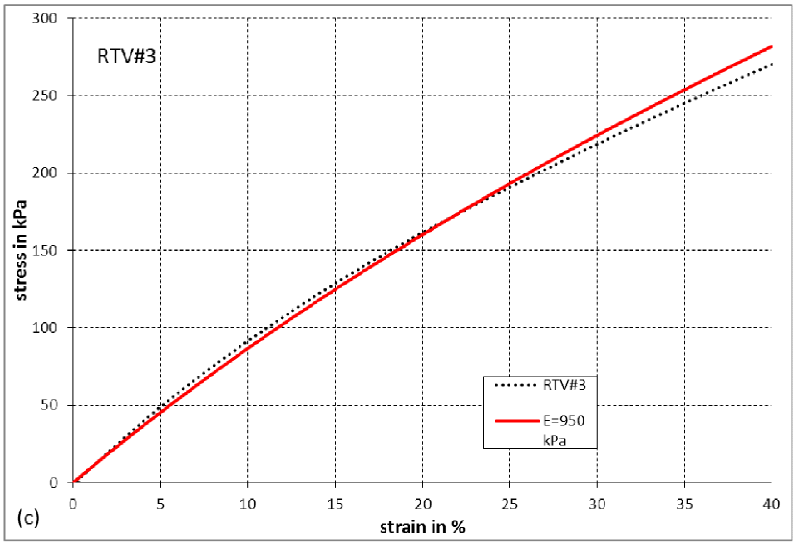
The RTV#2 measurements were similarly conducted. Again, the measurements at different strain rates showed no viscosity influence for this material. The Neo Hookean law fitting estimated the Young modulus to be 25 kPa.

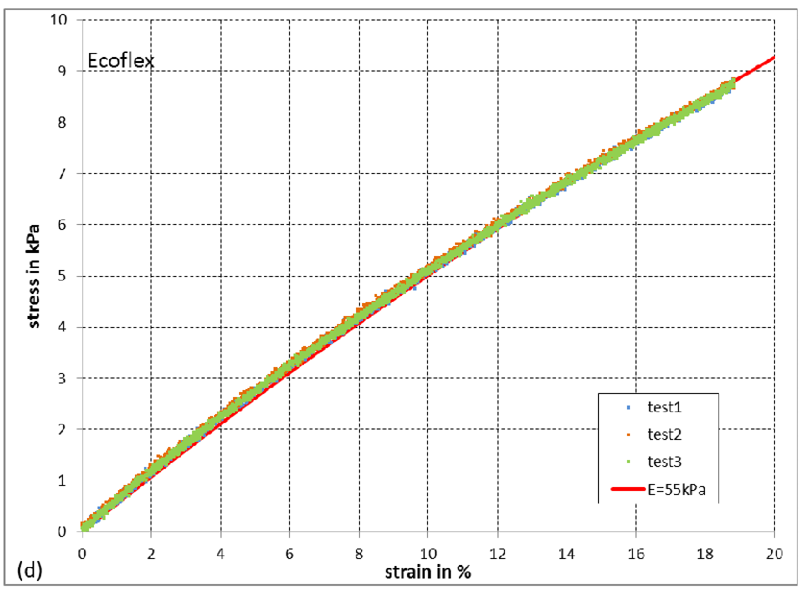
The measurements on the RTV#3 were highly reproducible and only one test is presented in Figure 6 c). A Young modulus of 950 kPa was estimated with the Neo Hookean law.

The measurements for Ecoflex estimated by the Neo Hookean constitutive law provided a Young modulus of 55 kPa. Finally, the tensile tests on the candle gel gave a Young modulus estimation of 10.5 kPa.



RTV#1
stress in kPa
30.00
25.00
20.00
15.00
10.00
5.00
0.00
0
5
10
15
20
25
30
35
40
strain in %
rtv1 slow
rtv1 fast
E=90kPa
(a)
RTV#2
stress in kPa
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
0
5
10
15
20
25
30
35
40
strain in %
rtv2 slow
rtv2 fast
E=25kPa
(b)


RTV#3
stress in kPa
strain in %
RTV#3
E=950 kPa
(c)


Ecoflex
stress in kPa
strain in %
test1
test2
test3
E=55kPa
(d)

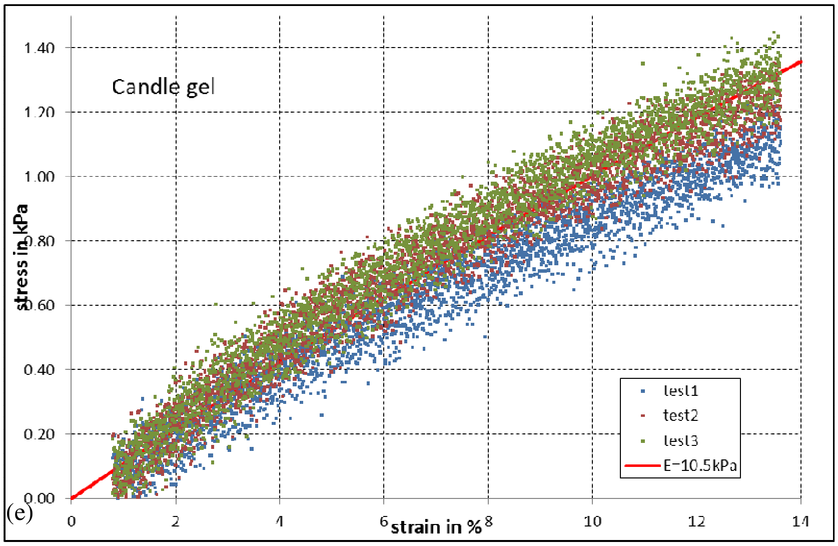


Figure 6 – Tensile measurements and Neo Hookean fitting for (a) RTV#1, (b) RTV#2, (c) RTV#3, (d) Ecoflex, and (e) candle gel.

Figure 7 summarizes the Young modulus estimated by the tensile test devices and by LASTIC. For the candle gel, a Young modulus of 10.5 kPa and 14 kPa were found for the tensile devices and LASTIC respectively, resulting in 33 % difference. Similarly, for the other materials, differences of 28 %, 22 % and 11 % were found for the RTV#2, Ecoflex, and RTV#1 respectively. It leads to an average 23.8 % ± 9.5 % SD overestimation of the Young modulus as given by LASTIC compared to the tensile tests.

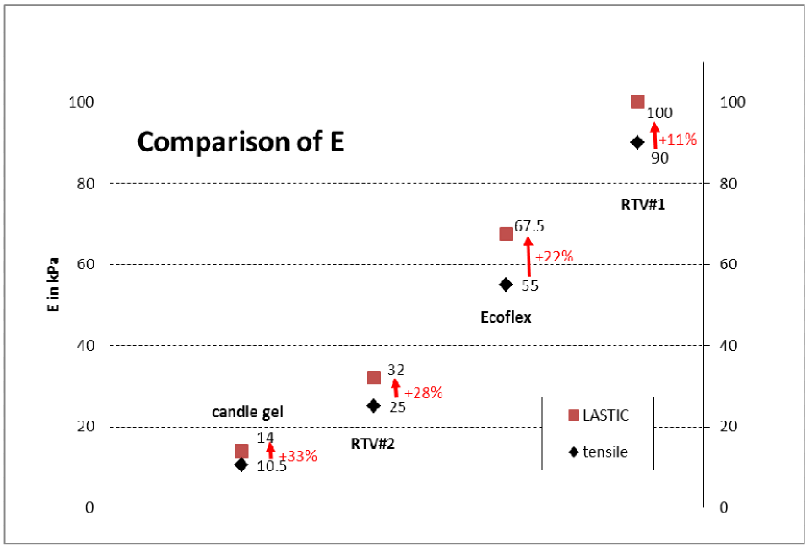


Figure 7 – Summary of the Young modulus found by LASTIC and the tensile devices for each material.

Figure 8 shows an example of the deformation of a sample using the finite element software ANSYS. ANSYS post processor was used to compute the Von Mises strains resulting from the negative pressure on the FE model. The higher values of the Von Mises strain naturally occur at the interface of the material and the inner edge of the vacuum chamber, where the sample is squeezed while the deformation dome is created. Observed strain in the central part of the model is around 20 % as shown on Figure 8 for a FEA simulating the RTV#2 silicone (For practical reasons, we used a Mooney Rivlin material for the simulation with $E = 32$ *kPa and* $\upsilon = 0.49$).

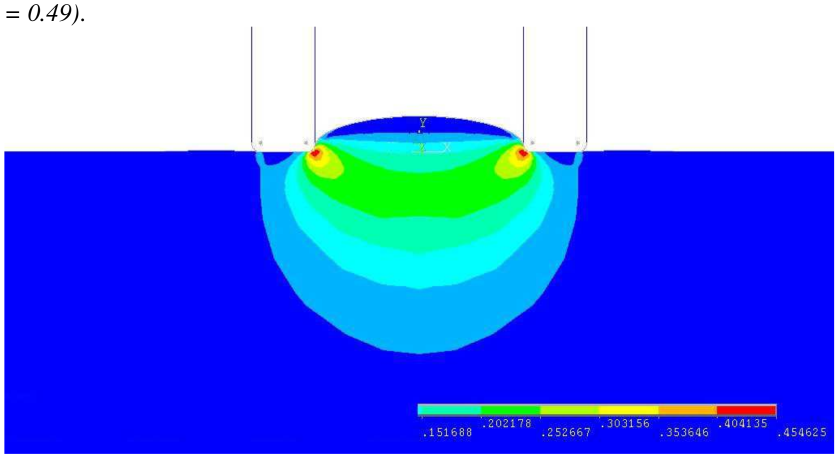


Figure 8 – Example of aspiration (Von Mises strain) of the RTV#2, using ANSYS FEA, with E = 32 kPa and υ = 0.49, with a negative pressure of -102 mbar. The aspiration appears at the center of the figure, between the two bars, representing LASTIC vacuum chamber.

4 Discussion

The average discrepancy of 23.8 % in the value of the Young modulus estimated between LASTIC and the tensile devices is quite high. However, this overestimation seems to be highly linearly dependent of the value of E. If this relatively constant inaccuracy was confirmed by further study, this could be taken into account during soft tissue characterization of living tissues. Nevertheless, for a precise estimation of the Young modulus, we should improve LASTIC by considering at least three main sources of error. Two sources of error are linked with the measurement devices, namely the manometer precision and the camera calibration, and the last one is due to the inverse problem solving.

The first area of possible improvement concerns the manometer. The digital manometer used in our set up has a precision of ± 6 mbar in measuring the negative pressure created with the pump (± 0.3 % of the full scale of the manometer range). Although rather small, this still represents 3 % of the maximum applied negative pressure for the RTV#1, 5 % for the Ecoflex, 7 % for the RTV#2, and 15 % for the candle gel. These variations could therefore be responsible for part of the total discrepancy. The fact that these variations are higher for the more elastic materials seems to corroborate our assumption that the manometer is responsible for part of the measurement deviation: a precision of ±6 mbar has more negative impact on a softer material. A more accurate manometer could help to reduce this error.

The second area for possible improvement is relative to the camera calibration. If the camera is exactly positioned on top of the mirror, with a point of view at 45 degrees, then the aspired distance measured as seen on the mirror exactly reflects the vertical aspired height. The incorrect orientation or angulation generates an additional perspective distortion that is not taken into account by our very simple manual calibration method which only considers the camera as an ideal pinhole. It should be possible to use a more sophisticated calibration procedure in order to better estimate the intrinsic as well as extrinsic camera parameters.

Because our simple calibration of the extrinsic parameter is mainly based on a pixel/mm ratio that depends on manually picked pixels corresponding to fiducial points inside the vacuum chamber, this ratio can be misestimated. Likewise, manual segmentation of the dome position can introduce errors in the Young modulus estimation. We are currently working on the development of a specific semi-automatic image segmentation algorithm that should decrease the dependency to manual picking. A second camera could be another solution since recording the aspiration from two viewpoints would provide a way to more accurately find the top of the deformation dome.

Finally, the third possible improvement could come from a better fitting of the negative pressure/apex displacement curves resulting from LASTIC by improving the FEM used to build the library of displacements. As shown on Figure 8, the observed strain in the central part of the model is around 20 %, which is in the same order as the strain applied with the tensile devices. Nevertheless, the highest strains appear in the elements that are at the interface with the vacuum chamber edges. Even if this is somehow expected, it can also indicate that the contact resolution is a crucial point and should be carefully monitored. Adding more elements or fine tuning the friction for these elements could also improve the FEA results and consequently the inverse problem resolution. Using a different nonlinear constitutive equation could also help in estimating the full scale of strains of the materials, whereas we are currently only focusing on the linear part of the strains. The FEM can also be improved: it is currently considering a pure symmetrical problem while the LASTIC's vacuum chamber hole is not centered, which might result in non-symmetrical load conditions. Avoiding the errors coming from the FEA could also be achieved by using an analytical solution such as the one presented in (Zhao et al., 2009). This kind of approach could give a more precise solution to estimate the Young modulus, avoiding approximation at the interface between the tissue and LASTIC device. Nevertheless, finding the proper analytical solution or an approximation could be a difficult task.

Another source of error in the estimation of the elasticity, not depending on LASTIC, could be coming from the tensile tests and their evaluation. These tests, while being the closest approximation available of the actual characteristics of the different materials, cannot be considered as gold standards. Some errors could be introduced while estimating the stress/strain curve of each material with a Neo Hookean constitutive equation. Furthermore, as shown in Figure 6, these measurements are not always reproducible and could also be the cause for some errors in the Young modulus estimation. This non reproducibility could be coming from the position of the samples between the tensile jaws which can sometimes be non-symmetrical, see Figure 4, and produce some slight errors in the measurements.

The impossibility to estimate the Young modulus of the RTV#3 by LASTIC measurements is mainly due to the high rigidity of this material: $E = 950$ kPa as estimated by the tensile test. Even with an aspired volume of 60 ml resulting in a negative pressure of 745 mbar, only 0.4 mm of apex displacement were measured with LASTIC, which is below the fixed threshold for an accurate estimation of the elasticity. Higher values of negative pressure are therefore needed to develop a more consequent aspiration height. Nevertheless, this was not possible with the type of pump used with LASTIC. Considering the range of elasticity found in living tissues, a limitation to linear elasticity lower than 1 MPa seems however to be acceptable.

5 Conclusion

This chapter presents a device, called LASTIC, to estimate the Young modulus of elastic materials by aspirating an area of their surface and recording the corresponding displacement. With Young modulus overestimated on average by 24 % compared to the value given by standard tensile devices, LASTIC needs to be improved before being used for accurate measurements. Nevertheless, because of the consistency in the overestimation and because of its small size, small cost and capability to be sterilized, LASTIC remains a good tool to estimate the elasticity of living tissues, even during surgery, in order to create a biomechanical simulation of these tissues. The previous version of LASTIC has already been used to characterize tissues of the skin, tongue, and brain, giving interesting insights and a good starting point for simulations.

Acknowledgments ANR TecSan IDS, Région Rhône-Alpes (projet SIMED, Cluster ISLE), Amandine Dufaug and Luc Maréchal (SYMME), Jérôme Giraud (LSP), Jacques Ohayon (TIMC).

References

Meunier L., Chagnon G., Favier D., Orgéas L., Vacher P. (2008) Mechanical experimental characterisation and numerical modelling of an unfilled silicone rubber, Polymer Testing, 27, pp.765-777.

Schiavone P., Boudou T., Ohayon J. & Payan Y. (2007). In-vivo measurement of the human soft tissues constitutive laws. Applications to Computer Aided Surgery. Computer Methods in Biomechanics & Biomedical Engineering, Supplement 1, pp. 185-186.

Schiavone P., Boudou T., Promayon E., Perrier P. & Payan Y. (2008). A light sterilizable pipette device for the in vivo estimation of human soft tissues constitutive laws. Proceedings of the 30th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, IEEE EMBS, pp. 4298-4301.

Schiavone, P., Chassat, F., Boudou, T., Promayon, E., Valvidia, F. and Payan, Y. (2009). In Vivo Measurement of Human Brain Elasticity Using a Light Aspiration Device. *Medical Image Analysis* 13:673-678.

Schiavone, P., Promayon, E. and Payan, Y. (2010). LASTIC: A Light Aspiration Device for in vivo Soft TIssue Characterization, *Biomedical Simulation: 5th International Symposium, ISBMS 2010* 5958:1-10.

Treloar, L. R. G. (1943). The elasticity of a network of long chains molecules I and II. Trans. Faraday Soc., 39, 236–246.

Vuskovic, V. (2001). Device for in-vivo measurement of mechanical properties of internal human soft tissues.

Zhao, R., Wyss, K., & Simmons, C. A. (2009). Comparison of analytical and inverse finite element approaches to estimate cell viscoelastic properties by micropipette aspiration. *Journal of Biomechanics*, *42*(16), 2768-2773.